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# Application of the NDHA model to describe $N_2O$ dynamics in activated sludge mixed culture biomass.

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**Abstract**
A pseudo-mechanistic model describing three biological nitric oxide (NO) and nitrous oxide ($N_2O$) production pathways was calibrated for an activated sludge mixed culture biomass treating municipal wastewater with laboratory-scale experiments.
The model (NDHA) comprehensively describes $N_2O$ producing pathways by both autotrophic ammonium oxidizing and heterotrophic bacteria. Extant respirometric assays and anaerobic batch experiments were designed to calibrate the endogenous, heterotrophic denitrification and autotrophic ammonium/nitrite oxidation processes together with the associated net $N_2O$ production. Ten parameters describing heterotrophic processes and seven for autotrophic processes were estimated accurately (variance/mean < 25%). The model predicted the $N_2O$ and NO dynamics at varying dissolved oxygen, ammonium and nitrite levels and was validated with a different set of batch experiments with the same biomass.
Aerobic ammonium oxidation experiments at two oxygen levels used for model evaluation (2 and 0.5 mg/L) indicated that the nitrifier denitrification (42, 64%) and heterotrophic denitrification (7, 17%) pathways increased and dominated the total $N_2O$ production at high nitrite and low oxygen concentrations; while the nitrifier nitrification pathway showed the largest contribution at high dissolved oxygen levels (51, 19%). The uncertainty of the biological parameter estimates was propagated to $N_2O$ model outputs via Monte Carlo simulations as 95% confidence intervals. The accuracy of the estimated parameters corresponded to a low uncertainty of the $N_2O$ emission factors (4.6 ± 0.6% and 1.2 ± 0.1%).
**Keywords**
Modelling; ASM; Nitrous oxide, Uncertainty, Activated sludge

## INTRODUCTION AND OBJECTIVES

$N_2O$ is a greenhouse gas emitted in wastewater treatment plants. In this study we aim to: (a) quantify $N_2O$ dynamics from mixed liquor biomass via extant respirometric assays, (b) calibrate the NDHA model to describe N-removing processes and $N_2O$ production and assess the accuracy of estimated parameters, (c) evaluate the predictive ability of the calibrated model against a different mixed liquor biomass and (d) quantify the uncertainty of $N_2O$ emissions during aerobic $NH_4^+$ removal.

## MATERIALS AND METHODS

*Model structure*

The NDHA was proposed as a consilient model to describe NO/$N_2O$ dynamics under a variety of conditions for biomass containing autotrophic and heterotrophic fractions (Domingo-Félez and Smets, 2016). Three biological pathways are considered: nitrifier nitrification (NN), nitrifier denitrification (ND) and heterotrophic denitrification (HD).

*Experimental design*

Respirometric approaches were taken (on-line, high-rate $O_2$ and $N_2O$ measurements) to obtain informative data on $N_2O$ dynamics from mixed liquor biomass (Table 1). Separate batch

nitrification experiments were executed in a 3-L lab-scale reactor with mixed liquor biomass from the same WWTP (Domingo-Félez et al., 2017b).

**Table 1** – Experimental design for lab-scale respirometric assays (A-C) and for model validation (D-E).

| Scenario | Oxygen level | Pulses | Monitoring | Targeted Processes |
|---|---|---|---|---|
| **(A)** | Anoxic | $NO_3^-$, $NO_2^-$, $N_2O$, COD | $NO_3^-$, $NO_2^-$, $N_2O$, $NH_4^+$ | Heterotrophic denitrification, hydrolysis |
| | From excess DO (air-sat) into anoxia | COD | DO | Biomass decay, hydrolysis |
| **(B)** | Anoxic | $NO_3^-$, $NO_2^-$ | $N_2O$, NO | HB-driven NO/$N_2O$ dynamics |
| **(C)** | From excess DO ($O_2$-sat) into anoxia | $NH_4^+$, $NH_2OH$, $NO_2^-$ | DO, $N_2O$, NO | $NH_4^+$, $NO_2^-$ removal<br>AOB/HB-driven NO/$N_2O$ dynamics |
| **(D)** | Constant aeration (high and low DO) | $NH_4^+$ | DO, $N_2O$, $NH_4^+$, $NO_2^-$ | $NH_4^+$, $NO_2^-$ removal, $N_2O$ dynamics |
| **(E)** | Constant aeration (high and low DO) | $NH_4^+$, $NO_2^-$, $NO_3^-$ | DO, $N_2O$, $NH_4^+$, $NO_2^-$ | $NH_4^+$, $NO_2^-$ removal, $N_2O$ dynamics |

*Sensitivity analysis, Parameter estimation procedure and Uncertainty analysis*

A global sensitivity analysis (GSA) was performed to identify the most determinant parameters on model outputs via Monte Carlo simulations using the SRC method. To test the validity of the model response the interdependency of residuals ($y_{sim,i}$ - $y_{obs,i}$) was analysed by autocorrelation for different lag times. The uncertainty of newly estimated parameters was compared to a reference case from literature and evaluated via Monte Carlo simulations (n = 500). More information can be found elsewhere (Domingo-Félez et al., 2017a).

## RESULTS

*Sensitivity analysis on a nitrification/denitrification case study*

Results from the GSA highlight the importance of AOB on $N_2O$ production from a mixed culture biomass during aerobic $NH_4^+$ oxidation. Up to four of the ten most sensitive parameters for $N_2O$ and NO liquid concentrations corresponded to AOB processes. Interestingly, NOB and HB were also sensitive, highlighting the importance of microbial interactions in complex communities.

*Biomass activity tests: example heterotrophic activity*

The specific denitrification rates and oxygen uptake rate were significantly higher in the presence of excess electron donor (mix of C-sources) compared to endogenous conditions: Denitrification (1.5, 2.5 and 4.7 vs. 7, 6.2 and 12 mgN/gVSS.h), C-removal (4.5-7 and 35 mgCOD/gVSS.h). The $N_2O$ reduction rate varied 3-fold in the pH range 6.5 - 9, with a maximum at around pH = 8 and lower rates towards higher and lower pH values (Figure 1).

*Calibration results: Heterotrophic and autotrophic N-removal*

Based on the overall good fit of model predictions and experimental data the NDHA model described the dynamics of the measured DO, $NH_4^+$, $NO_2^-$, $NO_3^-$, $N_2O$ and NO ($R^2 \geq 0.94$, F-test = 1 for 10/11 datasets). A total of 17 parameters were estimated with bounded approximate confidence regions indicating good identifiability (CV < 25%) (Figure 1, Table 2).

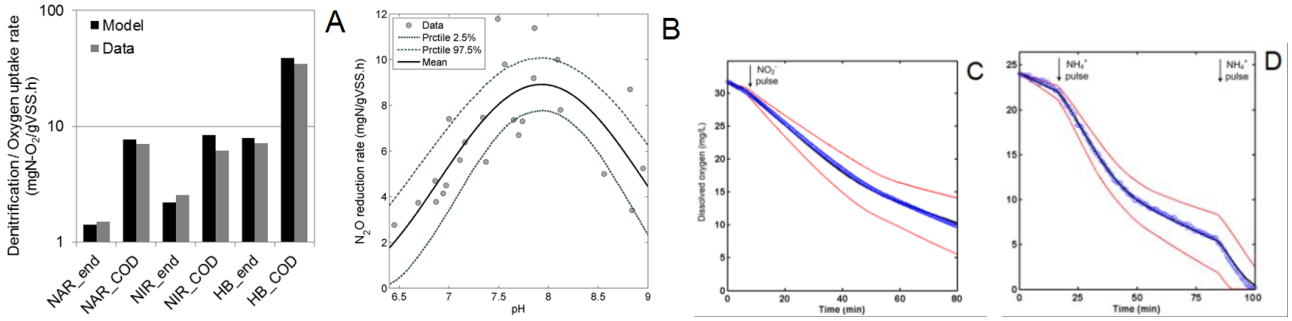


**Figure 1 –** Experimental and modelling calibration results for heterotrophic processes (A), nitrous oxide reduction dependency on pH (B), $NO_2^-$ oxidation (C), $NH_4^+$ oxidation (D). Red lines: 95% confidence intervals.

*Model evaluation*

The predictive ability of the calibrated NDHA model was evaluated on a set of batch experiments where mixed liquor biomass from the same WWTP was subject to varying N pulses at constant aeration. The model captured the trends of DO, main N-substrates and liquid $N_2O$ without any

**Table 2** – Best-fit values for the parameters estimated (25 °C).

| Parameter | Units | Value | Scen. | Parameter | Unit | Value | Scen. | Parameter | Unit | Value | Scen. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $pH_{opt.nosZ}$ | ( - ) | 7.9 ± 0.1 | (A) | $K_{HB.S.NIR}$ | mgCOD/L | 4.3 ± 0.69 | (B) | $\mu_{NOB}$ | $d^{-1}$ | 1.51 ± 0.07 | (C) |
| $w_{nosZ}$ | ( - ) | 2.2 ± 0.2 | (A) | $K_{HB.S.NOR}$ | mgCOD/L | 5.3 ± 0.83 | (B) | $\mu_{HB}$ | $d^{-1}$ | 7.23 ± 0.16 | (A) |
| $K_{HB.N2O}$ | mgN/L | 0.078 ± 0.020 | (A) | $K_{HB.S.NOS}$ | mgCOD/L | 4.1 ± 0.40 | (B) | $\varepsilon_{AOB}$ | ( - ) | 0.0031 ± 0.00 | (C) |
| $\mu_{HB.NAR}$ | $d^{-1}$ | 1.71 ± 0.11 | (A) | $K_{AOB.NH3}$ | µgN/L | 7.00 ± 1.17 | (C) | $\eta_{NIR}$ | ( - ) | 0.22 ± 0.01 | (C) |
| $\mu_{HB.NIR}$ | $d^{-1}$ | 1.11 ± 0.07 | (A) | $K_{NOB.HNO2}$ | µgN/L | 0.027 ± 0.006 | (C) | $\eta_{NOR}$ | ( - ) | 0.36 ± 0.02 | (C) |
| $\mu_{HB.NOS}$ | $d^{-1}$ | 1.17 ± 0.02 | (A) | $\mu_{AOB.AMO}$ | $d^{-1}$ | 0.86 ± 0.02 | (C) | | | | |

parameter modification ($R^2_{avg}$ for DO = 0.98; $NH_4^+$ = 0.99; $NO_2^-$ = 0.84; $N_2O$ = 0.80). Only the $N_2O$ residuals ($y_{sim,i}$ - $y_{obs,i}$) did not pass the F-distribution test ($F_{N2O}$ = 0). Higher $NH_4^+$ pulses yielded a higher $N_2O$ fraction (0.6 - 1.7 - 2.5 – 3.2% $N_2O/NH_4^+{}_{rem}$) as more $NH_4^+$ oxidation occurred at low DO, thus promoting the contribution of denitrification pathways (Figure 2).

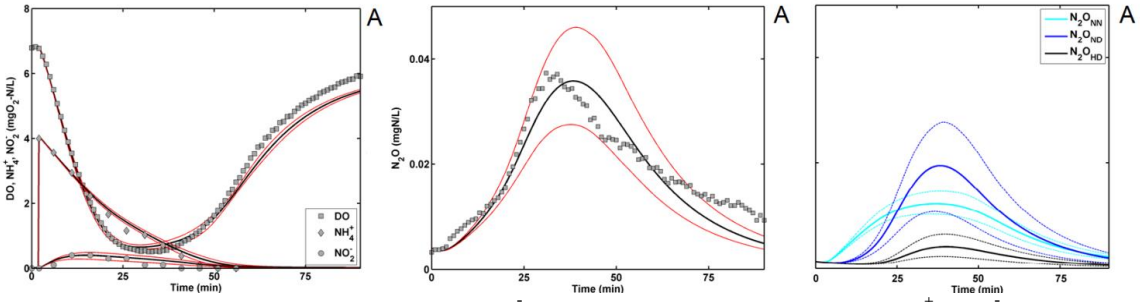


**Figure 2 –**Model evaluation results. Effect of $NO_3^-$ pulse (A). Main substrates: DO, $NH_4^+$, $NO_2^-$(left), $N_2O$ (middle) and $N_2O$ pathway contributions (right). Experimental results (markers), best-fit simulations (black lines), 95% confidence intervals (red lines) (left, middle). NN (cyan), ND (blue), and HD (black) pathway contributions.

To gain more insights on the $N_2O$ emissions from mixed liquor biomass during aerobic $NH_4^+$ oxidation simulations with best-fit estimate parameters were run for a wider range of DO (0.2 – 4 mg/L) and $NO_2^-$ (0 – 1.4 mgN/L) at excess $NH_4^+$. The $N_2O$ emission factor and individual pathway contributions to the total $N_2O$ pool at pseudo-steady state are shown in Figure 3.

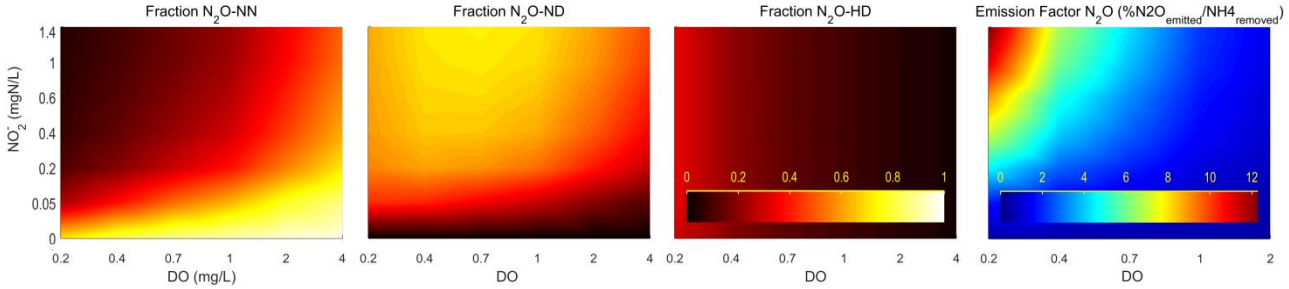


**Figure 3 –** Model evaluation at varying $NO_2^-$ and DO concentrations during excess $NH_4^+$ removal (pH = 7.2). From left to right: Pathway contributions to total $N_2O$ pool NN, ND, HD; $N_2O$ emission factor.

*Uncertainty of $N_2O$ emissions from activated sludge biomass*

The $N_2O$ emission factors of simulated $NH_4^+$ removal at constant DO (0.5 and 2.0 mg/L) are comparable to Wunderlin *et al.* (2012) at the same DO levels (3.8 and 2%, Table 3). The uncertainty of $N_2O$ model predictions was evaluated and could be used in future studies to discriminate between calibration procedures. By comparing the uncertainty of two cases (from a reference value from literature and the one obtained in this study) the uncertainty estimated with

this calibration procedure was only 28% of that simulated with the reference (Table 3). Here we show the impact of the uncertainty of biological parameter estimates in $N_2O$ emissions, which will significantly impact the carbon footprint of the process. Unfortunately, no other studies exist on uncertainty of $N_2O$ emissions. We believe that future comparison of best-fit simulations together with their uncertainty (e.g. 95% CI) will improve calibration procedures for $N_2O$ models.

**Table 3** – Nitrogen removal, $N_2O$ emission factor and $N_2O$ pathway contribution for the nitrification/denitrification case study after model calibration. The standard deviations (std) correspond to the uncertainty from estimated parameters in this study ($std_{t.s.}$), and a reference value from literature ($std_{init}$) for 500 Monte Carlo simulations.

| | | | $N_2O_{pathway\ contrib}$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **DO** | **ΔTN (mgN/L)** | **$N_2O_{emitted/removed}$** | **NN** | $std_{init}$ | $std_{t.s.}$ | **ND** | $std_{init}$ | $std_{t.s.}$ | **HD** | $std_{init}$ | $std_{t.s.}$ |
| 0.5 mg/L | 16.8 ± 0.1 | 4.6 ± 0.6% | 19% | 11% | 2% | 64% | 11% | 2% | 17% | 7% | 2% |
| 2.0 mg/L | 27.1 ± 0.3 | 1.2 ± 0.1% | 51% | 15% | 3% | 42% | 14% | 3% | 7% | 4% | 2% |

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